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Award Number: W81XWH-06-2-0052

TITLE: Genomic Typing of Red Cell Antigens

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REPORT DATE: **September 2011**

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Material Command Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
November 2011	Final	Aug 01, 2006 to Aug 31, 2011

4. TITLE AND SUBTITLE
Genomic Typing of Red Cell Antigens

5a. CONTRACT NUMBER
06-2-0052

5b. GRANT NUMBER
W81XWH-06-2-0052

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Puget Sound Blood Center
921 Terry Avenue
Seattle, WA 98104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES
Genomic typing, DNA, red blood cell antigens

14. ABSTRACT

Accurate typing for Blood groups is pivotal for successful transfusion. Historically serological reagents have shown to provide reliable typing for various blood groups within individual ethnic groups [1]. However, several populations have not adequately typed and gene frequencies of various blood types have not been established. Typing reagents are not universal as different ethnic groups show subtle nucleotide sequence variations accounting for the polymorphic nature of the blood types, in addition to varying in the gene distribution among populations [2-8]. To understand the serological disparities at molecular level we have undertaken side by side comparison of serological and molecular typing. This will facilitate development of various molecular methods for a rapid and accurate typing of blood groups.

15. SUBJECT TERMS
Transfusion Medicine, Blood Groups, Ethnic Groups and Molecular methods.

16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	U	25	USAMRMC
					19b. TELEPONE NUMBER (*Include area code)*

Standard Form 298 (Rev. 8-98) Prescribed by ANSI-Std Z39-1

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Introduction:

Critical to successful transfusion is the phenotyping of the donor and the recipient to ensure that the transfusion will be compatible. Historically serological reagents have shown to provide reliable typing for various blood groups within individual ethnic groups. As dependable as the hemagglutination methods have been for nearly 100 years, their reliance on typing sera subject them to inherent limitations that are both qualitative and quantitative in nature. While qualitative limitations hamper adequate typing for rare blood groups, variants, ethnic diversity and multiply transfused, the quantitative difficulties drive the price of reagents high. The subjective nature of the test performance and data interpretation could also have serious repercussions. The cumulative effect of all these factors is inadequate typing resource that slowly but surely becoming a burden on the healthcare [1].

This study was designed to set up genomic typing of red blood cell antigens and evaluate red cell antigens in populations which have not been well-studied. This is an important piece of information to determine the feasibility of performing red cell antigen typing using genomic tests universally. Genotyping for blood group polymorphisms is a work in progress as many laboratories are working diligently to include some sort of molecular assay for accurate typing. The genetic variation within a population, and across ethnic groups needs to be weighed in heavily when molecular typing assays are to be set up.

To address this, we had analyzed 10000 blood donors recruited from populations in the Pacific Northwest served by our blood center. These samples also cover African American donors from the Pacific Northwest. The frequencies of the genetic variants in these populations were determined and we had retained and samples of rare variants to provide reference material for future assay development.

Serious contenders for commercial kits for genotyping have not yet emerged that could even remotely address this. This makes our goal tougher to genotype the selected population from the Northwest, thus requiring us to establish appropriate genotyping methods along the way. Currently, amplification of selected regions followed by nucleotide sequencing was found to be most effective way of genotyping. However, we have identified primer sets to accomplish quick typing via PCR-SSP using 24 sets of primers.

BODY

Data collected and data analysis:

In previous quarterly reports, we had shown how systematically we had proceeded with few blood groups at a time and from each blood group we had tackled few polymorphisms at a time. We had used genotyping kits (BioArray Solutions) along with nucleotide sequence analysis demonstrated that the commercial kits are not completely ready for genotyping and additional probes needed to perfect the existing kits. We had also demonstrated that our sequence method along with procedures such as multiplex PCR allows us superior typing ability at a reasonable expense. However, these methods need to be automated and had to be packaged in some kind of array technology, which was beyond the scope of time and funding for this

project. However, the following data bullets will show what we had accomplished including a quick PCR-SSP method for cheap home grown kit.

- Completed recruitment and typing a total of 10,000 donors.

  Table 1: Ethnic breakdown of samples typed

Ethnicity	Number typed to date
API	2207
AS	5035
NA	1136
Blacks	1623
Total	**1001**

- Currently typing available for the following blood groups to identify corresponding number of polymorphic regions.

  Table 2: Blood groups with polymorphic sites that can be typed by nucleotide sequencing methods

Blood group	Polymorphic sites for which typing is available
ABO	47 SNP/InDels
FUT1 (Hh)	28 SNPs
FUT2 (Se)	16 SNPs
MNS System -	
MN (GYPA) and Ss (GYPB)	5 SNP/InDels
RH System -	
RHCE, RHD, RHAG	250 SNP/InDels
Lutheran (LU) – BCAM	2 SNPs
Kell (KEL)	10 SNPs
Duffy (FY) – DARC	6 SNPs
Kidd (JK) – SLC14A1	19 SNP/InDels
Diego (DI) – band 3 – SLC4A1	31 SNPs
Yt (YT) – ACHE	1 SNP
Scianna (SC) – ERMAP	6 SNP/InDels
Dombrock (DO) – ART4	14 SNP/InDels
Colton (CO) – AQP1	1 SNP
Landsteiner-Wiener (LW) – ICAM4	1 SNP
Gerbich (GE) – GYPC	3 SNPs
Cromer (CROM) – CD55 – DAF 2	8 SNPs
Knops (KN) – CR1 – CD35	5 SNPs
Indian (IN) – CD44	2 SNPs
Ok (OK) – BSG	1 SNP

Gil (GIL) – AQP3	1 SNP

- **Multiplex PCR developed to conserve time and resources**.

Amplicons from the multiplex PCR were sequenced and compared to individual sequencing. Thus, we have standardized nucleotide sequencing technology for routine typing of the following blood groups. The method need to be adapted to one of the rapid (possibly high throughput) technologies to improve the productivity.

Table 3: Multiplex PCR for RH and other blood groups. Currently these can be amplified in 6 different tubes. There are some that still need to be assessed as which group they can be worked into. All primer sets function well in individual amplifications. Currently, we are able to type any one or all of these blood groups by nucleotide sequencing. Especially, ABO and Rh are crucial as the commercial kits are lacking in reagents to cover all the variation. Our ABO typing includes FUT1 and FUT2 to be able to type Bombay and Para Bombay. We had provided several examples in our quarterly reports.

	Fwd Primer	Rev Primer	Multiplex Group
1	JK-E3-FP1	JK-E4-RP1	I
2	JK-I4-FP2	JK-I5-RP2	I
3	JK-I6-FP2	JK-I8-RP1	I
4	JK-E6-FP1	JK-I6-RP2	I
5	JK-I8-FP1	JK-I9-RP1	I
6	FUT-FP1A	FUT-E1-RP2	I
7	FUT2-FP1	FUT2-RP1	I
	FUT3-5pNCR-FP1	FUT3-E1-RP2	TBD
	FUT3-E1-FP4	FUT3-3pNCR-RP1	TBD
8	DI-E18-FP1	DI-E18-RP1	I
9	DO-I1-FP1	DO-2R	II
10	ABO-I3-FP5	ABO-I4-RP4	II
11	ABO-I5-FP1	ABO-I6n17986*	II
12	ABO-I6-FP1	ABO-3PNCR-RP1	redesign
13	FY-I1-FP2	FY2A-RP4	II
14	FY2B-FP3	FY-3NCR-RP2	II
15	FY-5NCR-FP1	FY-I1-RP2	II
16	SC-I1-FP2	SC-I2-RP1	II
17	GYPA-I1-FP2B	GYPA-I2-RPD	II
18	GYPB-I3-FP3	GYP(B)-I4-RP3B	II
19	CF-F-230/GYPBS-Int5	CF-R-230/GYPBS-Int5	NA
20	Gil-E5-FP2	Gil-I5-RP1	II
21	LU-I2-FP3	LU-I3-RP1	III
22	LU-I10-FP1	LU-I13-RP1	III
23	Co-E1-FP1	Co-E1-RP1	III
24	LW-E1-FP1	LW-E1-RP1	III
25	KEL-I5-FP2	KEL-I6-RP2	III
26	Kel-E7-FP1	Kel-I8-RP1	III
27	Kel-I16-FP2	Kel-I17-RP2	III
28	Kel-I15-FP2	Kel-I16-RP1	III
29	YT-E2-FP3	YT-I2-RP2	NA
30	XK-5pNCR-FP1	XK-I1-RP2	redesign
31	XK-I1-FP2	XK-I2-RP2	redesign
32	XK-I2-FP2	XK-3NCR-RP1	redesign

33	In-I1-FP2	IN-I2-RP1	NA

	Fwd Primer	Rev Primer	Expected Amplicon Size (bp)	Multiplex Group
1	RHDCE-5pNCR-FP1	RHCE-I1-RP1	200	RHCE
2	RHCE-I1-FP1	RHDCE-I2-RP2	200	
3	RHCE-I2-FP1	RHCE-I3-RP1	500	
4	RHCE-I3-FP2	RHCE-I4-RP1	265	none
5	RHCE-I4-FP1	RHCE-I5-RP1	661	RHCE
6	RHDCE-I5-FP1	RHCE-I6-RP2	170	
7	RHCE-I6-FP1	RHCE-I7-RP1	558	
8	RHCE-I7-FP1	RHDCE-I8-RP1	400	
9	RHCE-I8-FP4	RHDCE-I9-RP1	300	
10	RHDCE-I9-FP1	RHCE-3'NCR-RP1	200	
11	RHDCE-5pNCR-FP1	RHD-I1-RP1	200	RHD
12	RHD-I1-FP1	RHDCE-I2-RP2	200	
13	RHD-I2-FP1	RHD-I3-RP1	500	none
14	RHD-I3-FP2	RHD-I4-RP1	265	RHD
15	RHD-I4-FP1	RHD-I5-RP1	578	
16	RHDCE-I5-FP1	RHD-I6-RP2	170	
17	RHD-I6-FP1	RHD-I7-RP1	558	
18	RHD-I7-FP1	RHDCE-I8-RP1	400	
19	RHD-I8-FP4	RHDCE-I9-RP1	300	
20	RHDCE-I9-FP1	RHD-3'NCR-RP1	200	

- **Novel allelic variants were identified for Kidd and ABO blood groups only.**

The ABO variants accounts to mostly variations in intronic region and for A2 and O blood groups that do not seem to alter the function. Hence these are perceived not important variations for function. We have identified four novel SNPs for Kidd blood group all of which will result in null phenotype **[9-12].**

Table 4: Novel Kidd SNPs resulting in JKnull phenotype

Nucleic acid	Amino Acid	Background
191G>A	R64Q	JKB
742G>A	A248T	JKA
359G>A	G120E	JKB
536C>G	P179R	JKB

These nucleotide sequences were submitted to GenBank

Development of qPCR method for detection of C/c polymorphism. In addition to the multiplex PCR, we had developed a qPCR method for detectomg C/c polymorphism. (***Table 5a: C/c polymorphism***)

The C/c polymorphism is linked to six nucleotide subsitutions in exons 1 and 2 of *RHCE* gene. Exons 1 of *RHD* and the c allele are identical, and nucleotide sequence of *RHD* exon2 and C allele are indistinguishable as well. Current molecular typing of C alleles is based on the detection of a specific109bp insert in intron 2 present in many C alleles. The

genotype	**N of RHD copies**	
	exon2	exon4
Cc Dd	2	1
Cc DD	3	2
cc Dd	1	1
CC DD	4	2

insert is absent in c alleles and *RHD.* However, some of C alleles lack the 109bp insert. This causes discrepancy between molecular and serological typing and requires additional and expensive serological testing. To solve this problem we have developed quantitative real-time PCR analysis that assesses copy number of RHD/C specific exon2 by comparing it to the copy number of RHD specific exon 4 (see table 5a).

In a blind test of 14 previously typed samples we were able to confirm the C/c status of these samples with 100% accuracy (see Table 5b).

Table 5b Genotyping results

ID:	RHD:	RHC:	Notes:
LC 1	DD	CC	
LC 2	d	cc	
LC 3	DD	Cc	
LC 4	DD	CC	
LC 5	Dd	cc	
LC 6	Dd	Cc	
LC 7	DD	CC	
LC 8	Dd	Cc	
LC 9	DD	cc	
LC 10	DD	CC	
LC 11	Dd	Cc	
LC 12	D+	Cc	RHD Zygosity unknown
LC 13	DD	CC	
LC 14	DD	Cc	

Light Cycler Results:

Sample	qPCR
LC1	C+c-
LC2	D-C-c+
LC3	C+c+
LC4	C+c-
LC5	C-c+
LC6	C+c+(1xD)
LC7	C+c-
LC8	C+c+
LC9	C-c+
LC10	C+c-
LC11	C+c+(1xD)
LC12	C+c+
LC13	C+c-
LC14	C+c+

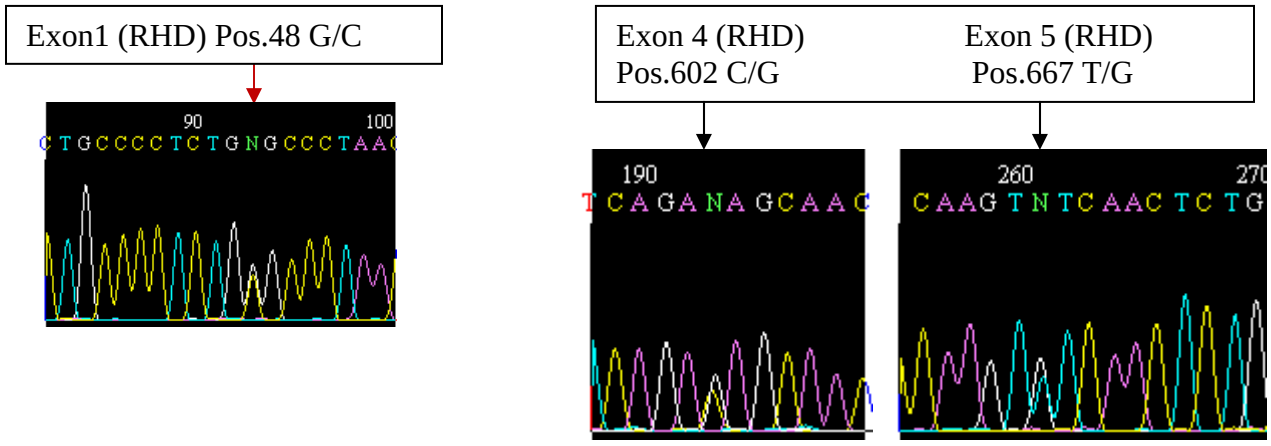
- **Ability to solve Rh discrepancies – example, a case report** (included in June 2011 report)

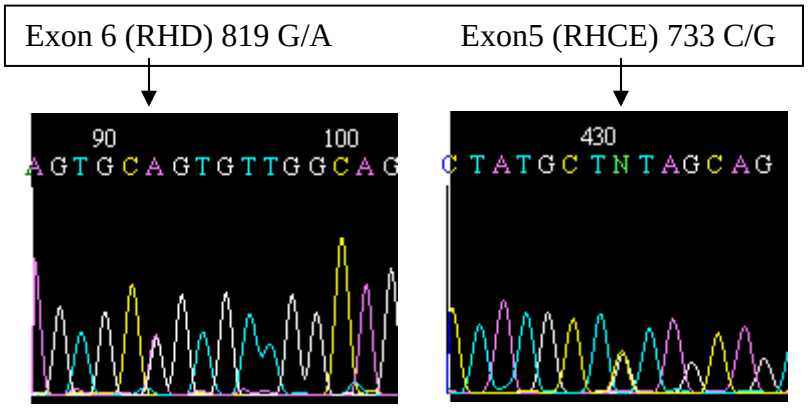
A pregnant woman who is D-negative by serology and father is serologically D positive. Two independent zygosity tests (RFLP of downstream RH-box and commercially available test from GTi Diagnostics) produced conflicting results with father's, mother's genomic DNA and DNA from amniocytes as well. We have been asked to figure out the cause of these discrepancies and to determine RHD copy number for father.

1. Father: (Electropherograms of various exons sequences are shown in Figure 1)

a) Gene copy-number q-PCR assay indicated that the father has two copies of exon4 RHD gene.

b) All 10 exons of the RHD and RHCE genes were PCR amplified and sequenced. Two RHD alleles were detected: *RHD*D/RHD*weak D type 4.1*(W16C, T201R, F223V) (48G/C, 602C/G, 667T/G, 819G/A). RHCE: *RHCE*RHCE/RHCE*RHce*^{s} (VS antigen 733 G).

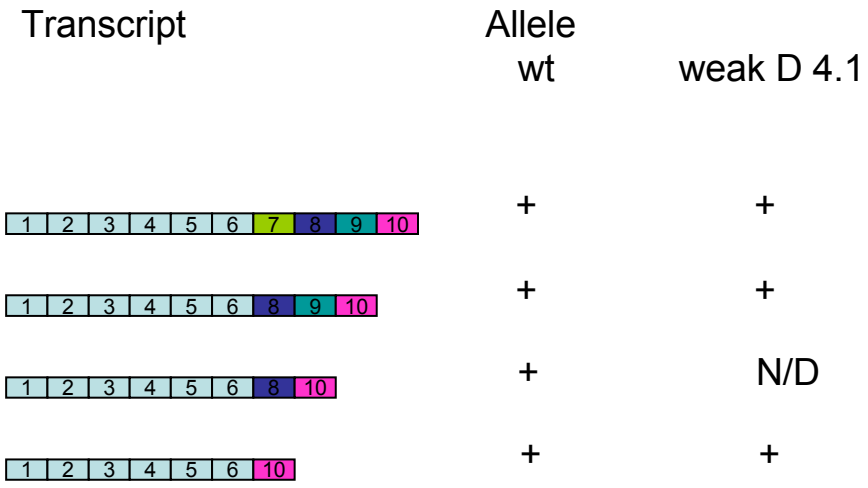




c) 3kb fragment of the downstream RH box was PCR amplified and sequenced. Multiple SNPs resembling nucleotide sequence of the upstream RH box were detected, including nt 5275 A/G that was generating an additional Pst I restriction site. Thus, observed polymorphism in the downstream RH box is the cause of discrepancy between RFLP and GTi zygosity tests.

d) RNA was isolated from RBCs, reverse transcribed and PCR amplified with RHD or RHCE sequence specific primers respectively. RHCE and RHD cDNA was sequenced directly. There was a 100% match between genomic DNA (coding exons of *RHCE*) sequences and RHCE transcripts.

Since the RHD mRNA consisted of a pool of multiple splice variants, RHD cDNA was subcloned into PCR2.1-TOPO vector and propagated in E.coli. DNA from 26 clones was isolated followed by sequencing. Figure 2 (below) shows that exons 7-9 of both normal and weak D 4.1 alleles were alternatively spliced.



2. Mother

a) No PCR fragments corresponding to RHD coding exons were detected, indicating *RHD*d/RHD*d* genotype. Sequencing of *RHCE* coding exons uncovered presence of VS antigen - carrying allele on heterozygous background: *RHCE*RHce/RHCE*RHce*^{s}.

b) Sequencing of the 3kb PCR fragment corresponding to the hybrid RH box revealed multiple SNPs similar to the SNPs detected in father's downstream box that most likely caused contradictory results of the two zygosity tests.

c) In agreement with gDNA sequencing results, two types of RHCE transcripts were detected corresponding to *RHCE*RHce* and *RHCE*RHce*^{s} alleles. No RHD specific transcripts were found, confirming deletion of *RHD* gene.

3. Amniocytes.

Due to a limited amount of gDNA, we were able to sequence coding exons of *RHD* and *RHCE* genes only. The observed genotype was: *RHD*weak D type 4.1(father)/RHD*d(mother); RHCE*RHce*^{s} */ RHCE*RHce*^{s}.

- Allelic frequencies of various blood groups among various ethnic groups were established (Table 6) (Gaur et al.: Manuscript in preparation). These were calculated from data derived by using the Beadchip from Bioarray solutions.

Table 6: Allelic frequency – population distribution Among Asian ethnic groups

		Chinese	Filipino	American Indian	Japanese	Korean	Native Alaskan	Pacific Islander/ Hawaiian	South Asian	Southeast Asian	Total
	N	1630	1236	873	981	959	252	453	879	893	8156
Colton											
	A+/B-	0.996	0.989	0.934	0.987	0.993	0.972	0.980	0.986	0.992	0.984
	A+/B+	0.004	0.010	0.065	0.013	0.007	0.028	0.020	0.013	0.008	0.016
	A-/B+	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A-/B-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All A	1.000	0.999	0.999	1.000	1.000	1.000	1.000	0.999	1.000	1.000
	All B	0.004	0.010	0.066	0.013	0.007	0.028	0.020	0.013	0.008	0.016
	***Inc**	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
Diego											
	A+/B-	0.000	0.000	0.000	0.001	0.003	0.000	0.000	0.000	0.001	0.001
	A+/B+	0.044	0.011	0.021	0.076	0.104	0.016	0.011	0.003	0.019	0.038
	A-/B+	0.954	0.988	0.978	0.922	0.892	0.976	0.989	0.997	0.980	0.961
	A-/B-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All A	0.044	0.011	0.021	0.077	0.107	0.016	0.011	0.003	0.020	0.038
	All B	0.998	0.998	0.999	0.998	0.996	0.992	1.000	1.000	0.999	0.998
	***Inc**	0.002	0.002	0.001	0.001	0.001	0.008	0.000	0.000	0.000	0.001
Kidd											
	A+/B-	0.218	0.211	0.246	0.234	0.210	0.226	0.287	0.315	0.280	0.242
	A+/B+	0.482	0.511	0.515	0.501	0.533	0.540	0.497	0.509	0.485	0.504
	A-/B+	0.299	0.278	0.238	0.264	0.258	0.234	0.214	0.176	0.235	0.253
	A-/B-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All A	0.700	0.722	0.762	0.735	0.742	0.766	0.784	0.824	0.765	0.746
	All B	0.781	0.789	0.754	0.765	0.790	0.774	0.711	0.685	0.720	0.757
	***Inc**	0.001	0.000	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.000

LW											
	A+/B-	0.999	0.996	0.994	1.000	1.000	0.988	1.000	1.000	1.000	0.998
	A+/B+	0.000	0.004	0.006	0.000	0.000	0.012	0.000	0.000	0.000	0.002
	A-/B+	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A-/B-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All A	0.999	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	All B	0.000	0.004	0.006	0.000	0.000	0.012	0.000	0.000	0.000	0.002
	***Inc**	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lutheran											
	A+/B-	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A+/B+	0.002	0.011	0.058	0.009	0.004	0.044	0.033	0.005	0.000	0.014
	A-/B+	0.997	0.988	0.940	0.991	0.996	0.956	0.965	0.994	1.000	0.986
	A-/B-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All A	0.002	0.011	0.060	0.009	0.004	0.044	0.033	0.005	0.000	0.014
	All B	0.999	0.999	0.999	1.000	1.000	1.000	0.998	0.999	1.000	0.999
	***Inc**	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.001	0.000	0.000
Kell											
	K+/k-	0.000	0.000	0.001	0.000	0.000	0.004	0.000	0.000	0.000	0.000
	K+/k+	0.002	0.011	0.057	0.014	0.004	0.067	0.029	0.014	0.008	0.016
	K-/k+	0.998	0.989	0.942	0.986	0.996	0.929	0.971	0.986	0.992	0.983
	K-/k-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	All K	0.002	0.011	0.058	0.014	0.004	0.071	0.029	0.014	0.008	0.017
	All k	1.000	1.000	0.999	1.000	1.000	0.996	1.000	1.000	1.000	1.000
	***Inc**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sciana											
	1+/2-	0.999	0.997	0.995	0.997	0.992	1.000	1.000	0.998	0.999	0.997
	1+/2+	0.001	0.002	0.005	0.003	0.004	0.000	0.000	0.002	0.000	0.002
	1-/2+	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	1-/2-	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.000	0.001	0.001
	All 1	1.000	0.998	1.000	1.000	0.996	1.000	1.000	1.000	0.999	0.999
	All 2	0.001	0.002	0.005	0.003	0.004	0.000	0.000	0.002	0.000	0.002
	***Inc**	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

***Inc** = Inconclusive

Diego shows ethnic variation which was presented earlier at AABB.

- **Rare Donors –**1291 donors of Asian descent were typed and frequencies for rare donors blood types were calculated (Table 7)

Table 7: Numbers of Rare donors from PSBC

No.	Rh Phenotype	Additional Antigen Combination	Frequency Per 1000 (Caucasian population)	Rare donors Identified (N=8157)	Frequency Per 1000 (Asian population)
1	R1R1	c- K- Fya- Jka-S-	7	19	2.3
2	R1R1	c-K-Fyb-Jkb-s-	1	10	1.2
3	R1R1	c-K-Fyb-Jka-s-	1	6	0.7
4	R1R1	c-K-Fya-Jkb-s-	2	1	0.1
5	R1R1	c-K-Fya-Jka-s-	2	5	0.6
6	R1R1	c-K-Fyb-Jkb-S-	4	476	58.4
7	R1R1	c-K-Fyb-Jka-S-	4	602	73.8
8	R1R1	c-K-Fya-Jkb-S-	8	24	2.9
9	rr	D-K-Fyb-Jkb-s-	1	4	0.5
10	rr	D-K-Fyb-Jka-s-	1	3	0.4
11	rr	D-K-Fya-Jkb-s-	1	2	0.2
12	rr	D-K-Fya-Jka-s-	1	1	0.1
13	rr	D-K-Fyb-Jkb-S-	3	8	1.0
14	rr	D-K-Fyb-Jka-S-	3	5	0.6
15	rr	D-K-Fya-Jkb-S-	6	5	0.6
16	R2R2	e-K-Fyb-Jka-S-	<1	53	6.5
17	R2R2	e-K-Fya-Jkb-S-	<1	4	0.5
18	R2R2	e-K-Fya-Jka-S-	<1	4	0.5
19	R2R2	e-K-Fyb-Jkb-s-	<1	2	0.2
20	R2R2	e-K-Fyb-Jka-s-	<1	0	0.0
21	R2R2	e-K-Fya-Jkb-s-	<1	0	0.0
22	R2R2	e-K-Fya-Jka-s-	<1	0	0.0
23	R2R2	e-K-Fyb-Jkb-S-	<1	57	7.0

- We were able to utilize the rare units identified in this study, by exporting them to various blood banks and hospitals in the country. (Table 8)

Table 8: Usage of rare units obtained from this study

Rare Factor	ABO/Rh	Ethnic Code	Last Donation	Dispensation
Jka-b-	A+	F	2/27/08	BBAK; Steven's
Jka-b-	B+	F	1/6/10	Frozen; Local; Expired
Jka-b-	O+	F	12/17/09	So Carolina; BBAK; Spokane; Cascade Reg.

Jka-b-	AB+	P	11/25/08	Local
Jka-b-	O+	P	7/9/10	So Carolina; BBAK; Cascade Reg.; Local
Jka-b-	A+	N	1/27/09	BBAK
Jka-b-	O+	P	10/18/09	Cascade Regional
Lub-	O-	I	4/30/08	Frozen
Lub-	O+	P	5/7/10	Cascade Valley
Dib-	O+	K	4/28/09	Local
Dib-	A+	K	7/30/09	Discarded (hemolyzed)
Dib-	A+	K	8/12/09	Skagit
Dib-	A+	K	2/12/10	Olympic Mem
Dib-	A+	J	11/22/08	Local
Dib-	AB+	T	7/8/10	Prov-Everett; Frozen
Coa-	O+	I	10/01/09	Frozen
k-	O-	I	2/5/10	Cent Illinois; BBAK; Local; Frozen
k-	O+	N	8/28/10	Lifestream; Cascade Valley; Local

- **Finally,** At the time of completion of the project – we left a quick comprehensive PCR-SSP testing for the clinically significant blood groups:
  Table 9: PCR-SSP for clinically significant blood groups

Pri Tube	System	Fwd Primer	Rv Primer	Temp	Size	Allele
1	Dombrock(Hy)	DO-I1-FP1	DO-E2-108G-p3*	60*	256	Hy+
2	Dombrock(Hy)	DO-I1-FP1	DO-E2-108V-p2*	60*	256	Hy-
3	ABO	ABO-E6-O-FP1	ABO-E6-RP1	60*	128	O
4	ABO	ABO-E6-AB-FP1	ABO-E6-RP1	60*	129	AB
5	ABO	ABO E7 235ser	ABO3A-E7-RP5	60*	240	B
6	Diego	Di-b-E18-854P1	Di-E18-RP1	60*	102	Dib
7	Diego	Di-a-E18-854L1	Di-E18-RP1	60*	102	Dia
8	Dombrock(a/b)	DO-E2-265D-p2	Do2R	62.5*	111	DoB
9	Dombrock(a/b)	DO-E2-265N-p3	Do2R	62.5*	111	DoA
10	Kidd(Jk a/b)	JK-Irshaid-FP1	Kidd 838G-Irshaid-RP1	62.5*	301	Jka
11	Kidd(Jk a/b)	Kidd 838A-Irshaid-FP1	JK-Irshaid-RP1	62.5*	121	Jkb
12	Kidd	JK-I4-AA-p2	JK-I5-RP1	62.5*	202	JK splice
13	Kidd	JK-I4-AG-p1	JK-I5-RP1	62.5*	202	JK spl WT
14	RHCE	RHcE-E2(1)=RHcE-E2p1	RHDCE-I2-RP1	62.5*	170	RHc
15	RHCE	RHC-109-FP1	RHC-109-RP1	62.5*	110	RHC

16	RHCE	RHE-FP1	RHEe-RP1	62.5*	145	RHE
17	RHCE	RHe-FP1	RHEe-RP1	62.5*	145	RHe
18	Colton	co-e1-fp1	CO-E1 45Ala-p3a*	64*	150	Coa
19	Colton	co-e1-fp1	CO-E1 45Val-p4a*	64*	150	Cob
20	Colton	co-e1-fp1	CO-E1 45Thr-p5a*	64*	150	Cob
21	Duffy	FY-I1-FP2	FY-E2-42G-p1*	64*	261	Fya
22	Duffy	FY-I1-FP2	FY-E2-42D-p2*	64*	261	Fyb
23	LW	lw-e1-fp1	LW-E1-100Gln-p1a*	64*	307	Lwa
24	LW	lw-e1-fp1	LW-E1-100Arg-p2a*	64*	307	Lwb

The primers and probe combinations identified to deliver an array based assay, that was beyond the scope of the allotted project budget or time line.

Key Research Accomplishments:

- Completed typing 10000 subjects (including 1639 African American) by commercial kits.
- **Ability to type for major and clinically significant blood groups with high accuracy.**
  1. We have developed primer sets that allow rapid amplification and nucleotide sequencing of about most of the blood groups (23 genes) including ABO, RHD and RHCE (Table 2-3)
  2. Multiple sections for each gene amplified to encompass all the polymorphic sites/SNPs known to alter antingenicity (Table 2)

- Identified and sequenced novel allelic variants and rare blood types from different ethnic group (Table 4)
- Able to genotype almost any blood group, including most of the RH alleles, by nucleotide sequencing (Tables 2- 5, figures 1-2)
- Able to get robust amplification for a multiplex – moved on to nucleotide sequencing in a multiplex set. (Table 3)
- Allelic frequencies were calculated for the blood groups available on the commercial beadchip (Table 6)
- Rare blood types were identified and distributed to various centers (7-8)

- Other functional assays:

1 RHD and RHCE
   a. Resolving weak D by genotyping was shown
   b. Zygosity assays developed

2 Functional assays for Kidd mutations that disrupt serological reactivity, were shown in previous annual reports.

3 There are few blood groups and few polymorphisms within these blood groups that are more crucial for a quick testing. Keeping this in view a rapid PCR-SSP was developed. This assay will take roughly 2 hrs and relatively inexpensive.

- **Provisional Patent application filed:** In the process of conducting this study, methods were established for genotyping and a provisional patent application has been filed, entitled "Compositions and Methods for Identifying Blood Group Polymorphisms"

Reportable Outcomes

Abstracts and publications:

Gaur, L., Posadas J, Teramura G, Degler J, Wood T, Gaur P, Haile A, Armour A, Nelson K. Novel Kidd Polymorphisms May Address Serological Discrepancies. Transfusion, 48 (Sup):13A, 2008.

Population distribution of Di^a and Di^b among Seattle Asian community. Lakshmi K Gaur, Jeffrey Posadas Gayle Teramura, Jackie Degler, Tara Wood, Prashant Gaur, Askale Hailey, Rosalind Armour, Karen Nelson Transfusion, 48 (Sup): 2008.

Feasibility of Providing Antigen-Matched Red Cells for Sickle Cell Anemia Patients Using Molecular Typing to Augment Testing: Meghan Delaney, Prashant Gaur, Askale Haile, Rosalind Armour, Lakshmi Gaur, Gayle Teramura. AABB (poster) 2009.

Gaur Lakshmi, Posadas J, Teramura G, Gaur P, Haile A, Devanaboina M and Nakaya, S. Molecular diversity of the jk null phenotype. XXXIst International Congress of the ISBT, Berlin, Germany, July 2010 (adjudged as top 10% best abstracts). Vox Sang. Vol 99 (suppl. 1) P-079.

Gaur Lakshmi, Gong B , Posadas J , Nester T, Reid,A , Armour R , Storry JR & Delaney, M. CASE REPORT OF TESTING AND MANAGEMENT OF BOMBAY (OH) PREGNANCY. XXXIst International Congress of the ISBT, Berlin, Germany, July 2010. Vox Sang. Vol 99 (suppl. 1) P-0855.

Posadas J, Shnyreva M, Gaur P, Gayle T, Haile A, Gaur LK: Distribution of JK silent alleles in Asian American and Pacific Islander Populations. AABB 2010 annual meeting, Oct 9-12, 2010, Baltimore, MD. Transfusion 50 (Suppl):143A, 2010.

Katrina L Billingsley, G Noumsi, J Posadas, L Gaur, J Moulds: Investigation of Molecular Typing Results Leads to the Identification of New JK Silencing Mutations in the African American Population. 2011 CBBS/SCABB Joint Meeting in Las Vegas (April 2011).

Manuscripts in preparation:

1. Gaur et al.: Distribution of JK-null alleles in Asian American Populations from the Pacific Northwest
2. Gaur and Deva: Novel ABO alleles.
3. Gaur et al: Distribution of JK silent alleles in Asian American and Pacific Islander Populations.
4. Gaur et al: Molecular Diversity Of The JK null Phenotype (Manuscript in preparation).
5. Delaney M et al: A Genetic Impossibility? Case report of Bombay (Oh) pregnancy with Group AB Infant (manuscript in preparation).

Provisional Patent application filed: A provisional patent application has been filed, entitled "Compositions and Methods for Identifying Blood Group Polymorphisms"

Conclusion:

We completed recruiting and typing 10,000 (>10,500) donors from Asian, Pacific Islander and Northwest Native Americans; this also includes a little over 1600 African American individuals. Data analysis Asian and Pacific Islanders is completed. This study comprises the largest data collection from one region comprising of multiple ethnic groups. Surprisingly, we had not found a large number of novel alleles as anticipated at the onset of the study. The large study did have other advantages and data points we did not anticipate. 1) We did discover some novel JK variants, which lead to serological disparities which may or may not result in functional disparities. However, these antigenic differences may be sufficient to cause hemolysis [9-13]. 2) The extensive analysis by nucleotide sequencing exposed areas of polymorphisms not covered in the commercial kits. This allowed us unravel yet undetermined sequences [9] as well as polymorphisms not covered by the commercial kits. 3) We have come up with improved set of polymorphic sets that would define the blood groups better. 4) Although, we had not identified novel alleles in large numbers, the data analysis yielded several rare allele combinations, some of which we were able to export to other blood centers and hospitals, across the country. We have identified several more rare units from our community recruited and hope this will continue for years to come. 5) Better testing methods for ABO and Rh with were not perfected in commercial kits was provided to Puget Sound Blood Center, prior to completion of this project. This project lays strong foundation to reason why genomic testing is important and provided clues to completing a robust test kit in collaboration with a commercial partner.

References

1. Giles CM: Three decades of reference serology. Transfus Med 1(3):145, 1991.
2. Hashmi G, Shariff T, Zhang Y, Cristobal J, Chau C, Seul M, Vissavajjhala P, Baldwin C, Hue-Roye K, Charles-Pierre D, Lomas-Francis C, Reid ME: Determination of 24 minor red blood cell antigens for more than 2000 blood donors by high-throughput DNA analysis. Transfusion 47(4):736, 2007.
3. Kormoczi GF, Wagner T, Jungbauer C, Vadon M, Ahrens N, Moll W, Muhlbacher A, Ozgul-Gulce S, Kleinrath T, Kilga-Nogler S, Schonitzer D, Gassner C: Genetic diversity of KELnull and KELel: a nationwide Austrian survey. Transfusion 47(4):703, 2007.
4. Daniels G: Blood group polymorphisms: molecular approach and biological significance. Transfus Clin Biol 4(4):383, 1997.
5. Vergnes H, Constans J, Quilici JC, Lefevre-Witier P, Sevin J, Stevens M: Study of red blood cell and serum enzymes in five Pyrenean communities and in a Basque population sample. Hum Hered 30(3):171, 1980.
6. Scott EM, Wright RC: Polymorphism of red cell enzymes in Alaskan ethnic groups. Ann Hum Genet 41(3):341, 1978.

7. Anstee D, Levene C, Mallory D, Overbeeke M, Poole J, Reid M, Smart E, Tani Y, Wendel S, Woodfield G: Rare blood. An ISBT Working Party report on rare blood donors. International Society of Blood Transfusion. Vox Sang 77(1):58, 1999.
8. Cohen T, Simhai B, Steinberg AG, Levene C: Genetic polymorphisms among Iranian Jews in Israel. Am J Med Genet 8(2):181, 1981.
9. Gaur, L., Posadas J, Teramura G, Degler J, Wood T, Gaur P, Haile A, Armour A, Nelson K. Novel Kidd Polymorphisms May Address Serological Discrepancies. Transfusion, 48 (Sup):13A, 2008.
10. Gaur Lakshmi, Posadas J, Teramura G, Gaur P, Haile A, Devanaboina M and Nakaya, S. Molecular diversity of the jk null phenotype. XXXIst International Congress of the ISBT, Berlin, Germany, July 2010 (adjudged as top 10% best abstracts). Vox Sang. Vol 99 (suppl. 1) P-079.
11. Posadas J, Shnyreva M, Gaur P, Gayle T, Haile A, Gaur LK: Distribution of JK silent alleles in Asian American and Pacific Islander Populations. AABB 2010 annual meeting, Oct 9-12, 2010, Baltimore, MD. Transfusion 50 (Suppl):143A, 2010.
12. Katrina L Billingsley, G Noumsi, J Posadas, L Gaur, J Moulds: Investigation of Molecular Typing Results Leads to the Identification of New JK Silencing Mutations in the African American Population. 2011 CBBS/SCABB Joint Meeting in Las Vegas (April 2011).
13. Horn T, Castilho L, Moulds JM, Billingsley K, Vege S, Johnson N and Westhoff CM. A novel JKA allele, nt561C>A, associated with silencing of Kidd expression. Transfusion, (online version Oct 24) 2011

Appendices

Appendix 1

Population distribution of Di^a and Di^b among Seattle Asian community. Lakshmi K Gaur, Jeffrey Posadas Gayle Teramura, Jackie Degler, Tara Wood, Prashant Gaur, Askale Hailey, Rosalind Armour, Karen Nelson Transfusion, 48 (Sup): 2008.

Background: The objective of the study was to identify potential blood donors for certain rare alleles. We have undertaken molecular typing analysis of various ethnic populations to identify rare variants and to determine the prevalence of these variants in our community. We have genotyped the donors for Diego, Dombrock, Duffy, Kell, Kidd, MNS and RHCE among others. We present the distribution of Di^a and Di^b from various Seattle area ethnic groups from a large population study comprising 2027 donors.
Method: We collected DNA from donors who had self-identified their ethnic background. The test sample is comprised of individuals from American Indian, Chinese, Filipino, Japanese, Korean, Hawaiian Pacific Islander, South Asian and Southeast Asian heritage. A HEA BeadChip™ (BioArray, Warren, NJ) was used to genotype each donor of interest and the data was compiled in a database for analysis.
Results: In a study of over 2000 donors, we confirm previous observations where the Asian populations have shown increased frequencies of Di^a. It was previously reported, Di^b is more common and Di^a was shown at variable levels in different populations; 12% Japanese, 11% Chippewa Indians and 5% in Chinese (Reid and Lomas-Francis. The Blood Group Antigen Facts Book. Elsevier Academic Press, 2004, p 302). In our study of 2017 subjects, we found individuals with Di(a+b+), Di(a+b-) and Di(a-b+) but not Di (a-b-) consistent with previous observations. We report the frequency of Di^b at over 99% for all groups studied. The incidence of Di^a recoded was highest in Seattle Korean group at 11%, followed by Japanese (6%) and Chinese (3%) [Table1].
Conclusion: Analyzing a large set of data with mixed ethnic makeup we confirmed higher incidence of Di^a in populations with Asian heritage. Among the Asian groups the Korean group showed the highest incidence for Di^a, almost twice that of the Japanese in our community.

Table 1: Molecular typing for Diego blood group was carried out. The ethnicities of the donors recorded as identified the donors. However, these may have to include mixed heritages. In a study of over 2000 donors, we confirm previous observations where the Asian populations have shown increased frequencies of Diego^a allele.

Ethnic code	total	A+/B+	A+/B-	A-/B+	A-/B-	All A+	All B+
A	427	3.3	0.5	96.3	0.0	3.7	96.3
B	5	0.0	0.0	100.0	0.0	0.0	100.0
D	1	0.0	0.0	100.0	0.0	0.0	100.0
F	277	1.8	0.7	97.5	0.0	2.5	97.5
I	219	0.0	0.5	99.5	0.0	0.5	99.5
J	323	6.2	0.0	93.8	0.0	6.2	93.8

K	225	11.1	0.9	88.0	0.0	12.0	88.0
L	2	0.0	0.0	100.0	0.0	0.0	100.0
N	67	3.0	0.0	97.0	0.0	3.0	97.0
P	117	0.9	0.9	98.3	0.0	1.7	98.3
S	181	0.6	1.7	97.8	0.0	2.2	97.8
T	191	2.1	1.6	96.3	0.0	3.7	96.3
	2035	3.5	0.7	95.8	0.0	4.2	95.8

Appendix 2

Gaur, L., Posadas J, Teramura G, Degler J, Wood T, Gaur P, Haile A, Armour A, Nelson K. ***Novel Kidd Polymorphisms May Address Serological Discrepancies.*** Transfusion, 48 (Sup):13A, 2008.

Background : In a parallel study comparing serological and molecular typing, we have encountered a few typing discrepancies. To resolve these discrepancies, we have undertaken nucleotide sequencing. Our main objective is to elucidate the nature of these discrepancies to improve current DNA testing while searching for allelic variants of the Kidd blood group. We present the nucleotide sequence data from the first few discrepant samples, which yielded interesting new polymorphisms.
Methods: We collected DNA and blood from donors various ethnic backgrounds in an attempt to identify rare alleles. Serological testing was done to determine Kidd blood group antigens. PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) and BeadChip (BioArray, Warren, NJ) assays were used to determine two SNPs (single nucleotide polymorphisms) at codon 280. To further confirm the results we have employed nucleotide sequencing of both coding and noncoding regions spanning over 18 kb (exons 3 to intron 9). Sequencing data was also used to search for previously reported mutations as well as others not reported.
Results : Nucleotide sequences for all the 10 samples were in complete agreement with data obtained in PCR-RFLP and HEA BeadChip (BioArray, Warren, NJ) assays. However, these assays primarily address codon 280 to discern between JKA and JKB. By employing extensive sequencing, we were able identify polymorphisms in 3 out of the 10 samples outside of what the two assays could identify. We were able to account for 2 of the discrepancies and found an unreported allelic variant: 1) heterozygous for the missense mutation T319M previously identified by Wester et al (Transfusion. 48(2):365-72, 2008) and was also heterozygous at codon 280; 2) heterozygous for an unreported missense mutation A248T and was also heterozygous at codon 280; 3) homozygous for the M167V mutation and was also heterozygous at codon 280. M167V and D280N have not been reported to be linked before.
Conclusion: The nucleotide sequence analysis revealed previously unreported polymorphisms which may be pivotal in defining the function of the molecule, and hence important to consider in future typing analysis to provide robust typing system.

Appendix 3

Meghan Delaney, Prashant Gaur, Askale Haile, Rosalind Armour, Lakshmi Gaur, Gayle Teramura. ***Feasibility of Providing Antigen-Matched Red Cells for Sickle Cell Anemia Patients Using Molecular Typing to Augment Testing:*** AABB (poster) 2009.

Background: Patients with sickle cell disease (SCD) have blood group phenotypes that are dissimilar to the Caucasian donor base. Chronic transfusions and a high incidence of red cell alloantibodies provides clinical impetus to shift compatibility testing to more complete matching. Molecular blood group analysis offers fast, reproducible means of typing donors and patients. We have typed 6000 minority donors since we moved from limited screening and on-demand serological typing to using a bead-chip array system (BioArray Solutions) for donor typing. To determine if the effort has yielded a base of antigen matched units for transfusion, we used SCD patients' red cell antigen profiles to conduct an inventory query. **Methods:** Each SCD patients' antigen profile was used to query the available red cell database using to identify antigen (and antibody) matching units; D, C, c, E, e, K, k, Jk^a, Jk^b, Fy^a, Fy^b, S, s. Profiles that had possible VS+V+ were required to get D- blood, as this Rh haplotype can have variant D expression. Duffy *FY*B* mutations identified in the silencing GATA box (-33C) were considered with and without Fy^{b-} to see if it facilitates finding appropriate blood since these patients rarely do make anti- Fy^b. All typing was done on the same molecular platform except D antigen which was done serologically. Donated units go into one database, thus some of the inventory had historical serological typing only. We searched available red cell units because it accurately reflects active donors. **Results:** Of the 46 SCD patients, all were D+, 62% GATA homozygote, 20% heterozygote (GATA+). Fifty-nine percent were lacking ≥6 of the 13 red cell antigens. Of these, 2.9 Fy^b- and 5.4 Fy^b+ mean units were available if GATA+. Thirteen had alloantibodies; of these 4.8 Fy^b- and 11.4 Fy^b+ mean units were available if GATA+. Eight were VS+V+; of these 0.4 Fy^b- and 7.4 Fy^b+ mean units were available if GATA+. Ten had 0 units when all antigens and antibodies were respected; however 8 had 6 mean units available when queried for Fy^b+ units. Two patients with 0 units were both s-. **Conclusions:** SCD patients have genetic blood group differences compared to the donor population. This inventory query provides evidence that most SCD patients' transfusion needs can be supported using antigen matched red cells. Knowing the GATA status increases the likelihood of finding units. The patients who remain at risk for having few units available are those requiring D- cells, such as possible VS+V+. This feasibility study adds to the growing body of evidence that molecular typing of blood donors is useful and recruitment of D- African American donors should be promoted.

Number of Negative Antigens	Number of Patient Profiles Percent (Number)	Mean Units Available If GATA+, Fy^b-	Mean Units Available If GATA+, Fy^b+
7	20% (9)	4	4.7
6	39% (18)	2.2	5.7
5	17% (8)	7.7	11
4	15% (7)	32.4	56.9
3	9% (4)	26	60.8
Total	100% (46)	72.3	139.1

Appendix 4

Gaur Lakshmi, Posadas J, Teramura G, Gaur P, Haile A, Devanaboina M and Nakaya, S. ***Molecular diversity of the jk null phenotype.*** XXXIst International Congress of the ISBT, Berlin, Germany, July 2010 **(adjudged as top 10% best abstracts**). Vox Sang. Vol 99 (suppl. 1) P-079.

BACKGROUND: The Kidd (JK) blood group contains two antithetical antigens, Jka and Jkb. The molecular basis for the polymorphism is due to a single transition (838G>A) resulting in an amino acid replacement (D280N) defining 3 common phenotypes Jk(a+b-), Jk(a-b+), Jk(a+b+) and a null phenotype, Jknull (a-b-) in which the RBCs lack the Jk antigen. Multiple mutations that cause Jknull phenotype have been described; they include a nucleotide transition at a splice site leading to loss of exon, nonsense, and missense mutations. In most cases, Jknull phenotypes are linked to specific ethnic groups or geographic origins.

AIM: 1). Identification and characterization of mutations resulting in Jknull phenotypes in Asian, Pacific Islander, Native American and Hispanic populations from the Pacific Northwest. 2). Differentiating silencing substitutions due to non surface expression from serological nonreactivity.

METHODS: Selected DNA from 106 donors were amplified for exons 3-4, 5, 6, 7-8, and 9 in 5 separate amplifications to cover all known SNPs. The amplification products were purified and nucleotide sequencing was performed. Full-length cDNA was synthesized in one step RT-PCR with JK (Kidd) specific primers. RT-PCR fragments were cloned into plasmid vectors and sequenced to determine backbone. Urea lysis assay was performed to test urea transporter activity whenever fresh red blood cells (RBCs) were available.

RESULTS: Seven different mutations were identified on either JKA or JKB backbones in 106 samples. Three of these were not previously described and all three were found closer to the putative transmembrane regions. Splice site mutations occurred in our study population with the greatest frequency. The others seem to occur in select groups. We have identified three novel mutations; R64Q (Japanese, Korean), P179R (Chinese) and A248T (American Indian). To evaluate expression and function of R64Q mutation, urea lysis assay was performed. The efficiency of red blood cell lysis in 2M urea was ~70%. RBCs of common Jk(a+b+), Jk(a+b-), Jk(a-b+) phenotypes was ~80%. Full length JK 1.2 kb cDNA was amplified using JK sequence specific primers confirmed expression of both JKA and JKB. cDNA was not available for the other two novel mutations.

CONCLUSION: There are multiple mutations responsible for Jk null phenotypes. In addition to previously described mutations, we have identified three novel mutations perhaps contributing to serological nonreactivity. It is also clear that there are qualitative differences among these variations. For example, in contrast to T319M (Wester, Transfusion, 2008. 48(2): p. 365-72), but similar to S291P (Sidoux-Walter, Blood, 2000. 96(4): p. 1566-73), the R64Q polymorphism did not drastically reduce urea permeability of red blood cells. The functional implication from this may not be clear as yet. However, it is indicative from our analysis that there are qualitative differences among variants which may be linked to specific geographic groups, needing further investigation.

Appendix 5

Gaur Lakshmi, Gong B , Posadas J , Nester T, Reid,A , Armour R , Storry JR & Delaney, M. ***CASE REPORT OF TESTING AND MANAGEMENT OF BOMBAY (OH) PREGNANCY.*** XXXIst International Congress of the ISBT, Berlin, Germany, July 2010. Vox Sang. Vol 99 (suppl. 1) P-0855.

BACKGROUND: The Bombay phenotype (Oh) is a rare red cell type that is characterized by the deficiency of H antigen on the surface of the red cell and anti-H in the serum. Although more than 30 different mutations in the FUT1 gene that give rise to Oh and partial Oh phenotypes in different populations, there are few reported cases of pregnancy outcomes in Bombay phenotype women to guide clinical management. This case report illustrates the clinical course and management of a pregnancy in a patient with Bombay phenotype.
AIM: To describe the obstetrical and transfusion medicine management of a woman with the Oh (Bombay) phenotype as well as genetic analysis of her and her infant's red cell FUT1, FUT2, and ABO genes.
METHODS: Throughout the pregnancy, maternal anti-H titers were monitored. The blood samples from the mother and baby (after delivery) were serologically tested for ABO blood group and DNA was extracted and amplified for specific regions of the ABO (exons 3, 6 and 7), FUT1 (H) and FUT2 (Se) genes. The amplified nucleic acid products were subjected to direct sequencing using the amplification primers, as well as cloned in TA cloning vector PCR-TOPO2.1 vector (Invitrogen, Carlsbad, CA). Confirmatory testing was done with PCR-SSP and PCR-RFLP.
RESULTS: Serial IgM and IgG anti-H titers remained stable. Autologous blood donations for a possible intrauterine transfusion and/or postpartum hemorrhage were not used. Delivery of a healthy female neonate was uneventful. The serologic studies showed the mother and baby's phenotypes as O and AB, respectively. Genomic typing demonstrated the maternal ABO genotype as BO1, and phenotype as Oh (Bombay) with anti-H. Mother was homozygous for FUT1 725G, a silencing mutation associated with the concomitant deletion of FUT2. The infant was heterozygous for the missense mutation 725T>G in FUT1, providing one functional allele for the H-transferase (α1,2-fucosyltransferase). The infant's ABO genotype is A1B and phenotype is AB, with no detectable plasma type at the time of testing.
CONCLUSION: The genotyping studies confirmed serological findings; the maternal ABO genotype is BO1, and phenotype is Oh (Bombay). Maternal plasma contained a strongly reactive anti-H, consistent with this phenotype. The infant's ABO genotype is A1B and phenotype is AB, with no detectable plasma type at the time of testing. This case illustrates the clinically benign nature of the anti-H antibody to an A1B fetus and demonstrates of the underlying functionality and inheritance of the H and ABO blood group systems. This work was supported by funds from US Armed forces (USAMRMC-BAA05-01).

Appendix 6

Posadas J, Shnyreva M, Gaur P, Gayle T, Haile A, Gaur LK: Distribution of JK silent alleles in Asian American and Pacific Islander Populations. AABB 2010 annual meeting, Oct 9-12, 2010, Baltimore, MD. Transfusion 50 (Suppl):143A, 2010.

Background:

Jka /Jkb proteins are main components of the Kidd blood group system. Currently splice site and missense mutations are identified as cause of serological non reactivity. In most cases, Jk null phenotypes are linked to specific ethnic groups or geographic origins. In this study we present a distribution of serological nulls/silent alleles found among Asian and Pacific Islander Americans residing in the Pacific Northwest.

Methods:

Samples were collected from volunteer blood donors, who self identified their ethnic makeup, at the Puget Sound Blood Center, Seattle with informed consent. JK phenotypes were resolved using monoclonal anti-JKa and anti-JKb antibodies. Genotyping was done by Beadchip™ and PCR-RFLP where a transition at nt 838G>A resulting in a single amino acid difference Asp280Asn differentiates JKA and JKB; in cases of discrepancies additional sequencing of whole JK gene was performed.

Results:

We have analyzed 6222 Asian donors and identified 102 serological nulls. Nucleotide sequencing of JK gene from these samples yielded 4 SNPs in the transmembrane domain region and a splice site mutation. Distribution of these silencing mutations show clear differences between the Mainland and Filipino & Pacific Islanders (See Table).

A high incidence (4%) of null phenotype was observed in Filipino and Pacific Islander population, and only splice site mutations were found (63:0) in this group. A more even distribution of transmembrane domain SNPs and splice site mutations (17:12) was seen in Mainland populations. In South Asians, the previously described T319M is more prevalent than splice site mutations. The two novel mutations, G120E and P179R, have been found only in Southeast Asian and Chinese populations, respectively. G299E was seen in Chinese populations as previously reported.

Conclusions:

In a large study of Asian/Filipin & Pacific Islander populations, we found an ethnic specific distribution of missense and splice site mutations resulting in Kidd serological nulls. While only splice site mutations were observed in the latter group, both missense and splice site mutations were found in the former.

Appendix 7

Katrina L Billingsley, G Noumsi, J Posadas, L Gaur, J Moulds: Investigation of Molecular Typing Results Leads to the Identification of New JK Silencing Mutations in the African American Population. 2011 CBBS/SCABB Joint Meeting in Las Vegas (April 2011).

BACKGROUND: To date, 14 polymorphisms causing Jk null phenotypes have been reported, two of which are associated with people of African ethnicity. Although these mutations were found in single individuals presenting with the Jk(a-b-) phenotype, it was suggested that they may cause discrepant Jk typings. Thus, to assess the occurrence of JK silencing mutations in the local African American (A-A) population, a comparative was conducted.
METHODS: A-A blood donors and sickle cell disease patients (n=250) were genotyped using HEA v1.2 (BioArray) and the results were compared to historical serological types. Discrepant results were confirmed by repeat serological typing or allele specific PCR (GTI). Unresolved discrepancies were sent for DNA sequencing.
RESULTS: A total of 1,186 donors had previous Jka serological typing results and 1,349 donors had previous Jkb results. A total of four Jka (0.3%) and 34 Jkb (2.5%) samples were found to be discrepant but the majority were due to serological mistyping or data entry errors. Only five of these samples (0.2%) were found to be truly discordant. All five were predicted to be Jk(a+b+) by genotyping, yet one sample was Jk(a-), while four samples were Jk(b-) by serology. DNA sequencing resulted in identification of JK silencing alleles. The silenced JK*A allele was due to a promoter region mutation, while all four of the JK*B silencing alleles were the result of a 64R>Q substitution. Thus, this JK*B null allele is fairly frequent occurring in 1:337 A-A donors. A retrospective review of patient testing also yielded iscrepancies; two were JK*A/JK*B by genotype and Jk(b-) by serology. Sequencing confirmed one patient positive for the 64R>Q substitution while the second patient appears to exhibit a currently undetermined mutation
CONCLUSION: While some microarray genotyping assays have incorporated JK null detection, these are usually limited to the more common Polynesian and Finnish mutations. Previously JK nulls have rarely been found in A-A. As shown by this study, antigen screening which uses only SNP analysis may not detect blood group mutations due to silencing mutations and may lead to erroneous testing results. This may be of concern for sickle cell disease patients for whom genotyped matched blood is being selected.